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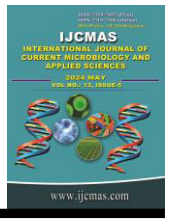

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Investigating the Anticancer Potential of *Eisenia fetida* Coelomic Fluid: A Comparative Study of Extraction Methods and Effects on Breast Cancer Cell Lines

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A B S T R A C T

Earthworm coelomic fluid has garnered attention for its potential anticancer properties, with studies primarily concentrated in China and India. This research investigates the extraction methods, protein concentration, and effects of coelomic fluid from *Eisenia fetida* on breast cancer cell lines MCF-7 and 4T1. The warm water shock and electric shock methods were compared, revealing differences in yield and protein concentration. While the electric shock method was more efficient in extraction, the warm water shock method yielded higher protein concentration. Both methods inhibited cell growth, with the warm water shock method exhibiting slightly superior results. Regression analysis showed concentration- and time-dependent responses, with optimal effectiveness observed at lower concentrations and within the initial 24 hours. Comparative analysis indicated comparable inhibitory effects between extraction methods. Our findings contribute to understanding the anticancer properties of earthworm coelomic fluid and its potential as a therapeutic agent. Previous studies have demonstrated its apoptotic induction of cancer cells and synergistic effects with chemotherapeutic agents. Further research is needed to explore its efficacy in clinical settings and potential in combination therapies.

Introduction

Cancer, characterized by the uncontrollable division and invasive behavior of abnormal cells, stems from damage to genetic material (Macdonald *et al.,* 2004; McGuire, 2016). Identifying the precise triggers of this damage often proves elusive (de Martel *et al.,* 2012). Key hallmarks of cancer cells include resistance to growth control mechanisms, evasion of cell death, insensitivity to growth inhibition signals, and stimulation of angiogenesis (Fiaschi and Chiarugi, 2012). Environmental factors predominantly influence cancer development, encompassing pollutants, carcinogens, mutagens, infectious agents, and genetic predispositions (Wu *et al.,* 2016; Danaei *et al.,* 2005).

Breast cancer, originating from breast cells, manifests as malignancy within breast tissues, notably in lobules and ducts (American Cancer Society, 2008; Nicholas Zdenkowski *et al.,* 2016). Symptoms encompass breast nodules, pain, and diverse metastases (Allred, 2010). Various factors, such as age, hormonal influences, and lifestyle, contribute to its incidence (Nelson, 2006). Treatment modalities, tailored to disease stage and

patient condition, include surgery, radiotherapy, hormone therapy, immunotherapy, and chemotherapy (Breast Cancer: What You Need To Know Before Treatment, 2003; Sudhakar, 2009).

Chemotherapy, employing drugs to impede cancer cell growth, entails collateral damage to healthy cells, precipitating diverse side effects (Irma and Beijnen, 2008; Porter, 2009).

Drug resistance poses a significant challenge, driving ongoing research for drug optimization and novel agents (Gottesman, 1993; Ambudkar *et al.,* 2003). Natural remedies, including earthworm-derived compounds, garner attention for their potential anticancer properties (Bernardini *et al.,* 2018; Sekhar *et al.,* 2018).

Historically used in traditional Chinese medicine, certain earthworm species, notably *Eisenia fetida*, exhibit therapeutic potential (Sun, 2015). Research on earthworms' anticancer properties, primarily concentrated in China and India, highlights their coelomic fluid as a promising avenue (Augustine *et al.,* 2018; Edwards and Bohlen, 1996).

Rich in eleocytes and fat droplet-containing cells, earthworm coelomic fluid demonstrates significant anticancer effects, including cell cycle inhibition and apoptosis induction (Urry *et al.,* 2015; Augustine *et al.,* 2019). Moreover, its antimicrobial properties offer additional therapeutic potential (Sethulakshmi and Lakshmi, 2018). Extraction methods, such as warm water shock or electric shock, yield this valuable fluid (Patil and Biradar, 2017).

Materials and Methods

Cell Lines

MCF-7 and 4T1 breast cancer cell lines were procured from the Pasteur Institute of Iran. MCF-7 represents luminal type A breast cancer cells, exhibiting characteristics akin to both normal breast epithelial cells and metastatic cancer cells (Dittmer *et al.,* 2011; Schweizer *et al.,* 2015). Notably, these cells display a robust proliferation rate and distinct nuclear morphology under laboratory culture conditions (Horwitz *et al.,* 1975). The 4T1 cell line, derived from spontaneous tumors in BALB/c mice, possesses high metastatic potential (Rathi, 2009; DuPré *et al.,* 2007).

Reagents

DMEM culture medium, streptomycin/penicillin solution, fetal bovine serum (FBS), trypan blue, trypsin, and MTT assay kit were procured from BIO-IDEA Company (Tehran, Iran). All other chemicals and reagents utilized were of analytical grade and commercially available.

Earthworms and Coelomic Fluid Extraction

Earthworms of the *Eisenia fetida* species were sourced from the Department of Life Sciences Engineering, Faculty of New Sciences & Technologies. These were nourished with composted cow manure, maintained at 70-80% moisture and 20-25°C temperature. Coelomic fluid from *Eisenia fetida* was extracted using warm water shock and electric shock methods (Patil and Biradar, 2017). Each extraction utilized 33 grams of earthworms, approximately equivalent to 100 adult specimens.

Determination of Total Coelomic Fluid Protein Concentration by Bradford Method

The Bradford method was employed to determine the total protein concentration of coelomic fluid extracted via warm water shock and electric shock methods, each at a concentration of 1 mg/ml (Bradford, 1976).

Cell Culture and Counting

MCF-7 and 4T1 breast cancer cell lines were cultured in DMEM medium supplemented with 20% FBS, 1% penicillin, and streptomycin under standard conditions (37°C, 90% humidity, 5% CO2). Upon reaching 80% confluence, cells were harvested, centrifuged, and resuspended in fresh culture medium. Cell viability was assessed by trypan blue exclusion method.

Determination of Cell Growth Inhibition Percentage by MTT Method

Cells were seeded in 96-well plates at a density of 5 x 105 cells per well and treated with coelomic fluid extracted via warm water shock and electric shock methods at concentrations of 0.25, 0.5, and 1 mg/ml. Viability was assessed using the MTT assay after 24, 48, and 72 hours of treatment. Absorbance was measured at 600 nm wavelength using an ELISA reader, and inhibition percentage was calculated relative to control OD.

Statistical Analysis

Statistical analysis was conducted using SPSS 24 software with four repetitions in a completely randomized block design. T-tests with unequal variances and Duncan tests were employed for mean comparison. Standard error depicted deviation from mean data, and Excel 2017 was utilized for graphical representation.

A regression model was applied to investigate the influence of time and concentration on cancer cell line inhibition percentage, with 24 hours, 0.25 concentration, 4T1 cell line, and electric shock method as baseline parameters.

Results and Discussion

Extraction of Coelomic Fluid

Comparison of the dry weight of coelomic fluid extracted by warm water shock and electric shock methods revealed a significant difference. The electric shock method yielded a larger quantity of coelomic fluid compared to the warm water shock method (Table 1).

The electric shock method demonstrated higher efficiency in extracting coelomic fluid, requiring fewer earthworms while yielding a greater volume of fluid.

Protein Concentration Measurement

Analysis of total protein concentration in coelomic fluid revealed variations between the warm water shock and electric shock methods (Table 2).

The warm water shock method resulted in higher total protein concentration compared to the electric shock method.

Effect of Coelomic Fluid on Cell Lines

Both warm water shock and electric shock methods produced coelomic fluid with inhibitory effects on MCF-7 and 4T1 cell lines across different concentrations and time intervals (Figure 1).

The inhibitory effect remained consistent across concentrations and time intervals, indicating a concentration-dependent and time-dependent response.

Regression Analysis

Increasing coelomic fluid concentration from 0.25 mg/ml to 1 mg/ml led to a moderate increase in inhibition percentage, suggesting optimal effectiveness at lower concentrations (Figure 2).

Similarly, extending exposure time from 24 hours to 72 hours resulted in a slight increase in inhibition percentage, indicating maximum efficacy within the initial 24 hours (Figure 3).

Comparative analysis between extraction methods demonstrated comparable inhibitory effects, with warm water shock method exhibiting slightly superior results despite potential drawbacks in earthworm viability (Figure 4).

Furthermore, MCF-7 cell line exhibited higher inhibition percentages compared to 4T1 cell line across both extraction methods (Figure 5).

Regression analysis confirmed the higher inhibitory potential of MCF-7 cell line compared to 4T1 cell line (Figure 6).

While both extraction methods yield coelomic fluid with inhibitory effects on cancer cell lines, the warm water shock method offers slightly superior results, emphasizing the importance of considering both efficacy and practicality in experimental design.

In recent years, numerous studies have explored the potential anticancer properties of coelomic fluid extracted from various species of earthworms. Several noteworthy investigations are highlighted below. Yanqin *et al.,* (2007) demonstrated the apoptotic induction of HeLa cancer cells by *Eisenia fetida* coelomic fluid.

Through meticulous experimentation involving coelomic fluid extraction and subsequent preparation of three concentration gradients, the researchers observed a dose- and time-dependent cytotoxic effect on cancer cells, as evidenced by MTT assays, AO/EB double staining, and agarose electrophoresis analysis of genome fragmentation (Yanqin *et al.,* 2007).

Table.1 Dry weight comparison of coelomic fluid extracted by two methods

Coelomic fluid extraction method	Dry weight (mg)
Warm water shock method	**120**
Electric shock method	**230**

Table.2 Total protein concentration of coelomic fluid extracted by two methods

Coelomic fluid extraction method	Total protein concentration (µg/ml)
Warm water shock method	**843**
Electric shock method	**429**

Figure.1 Percentage of growth inhibition by concentrations of coelomic fluid

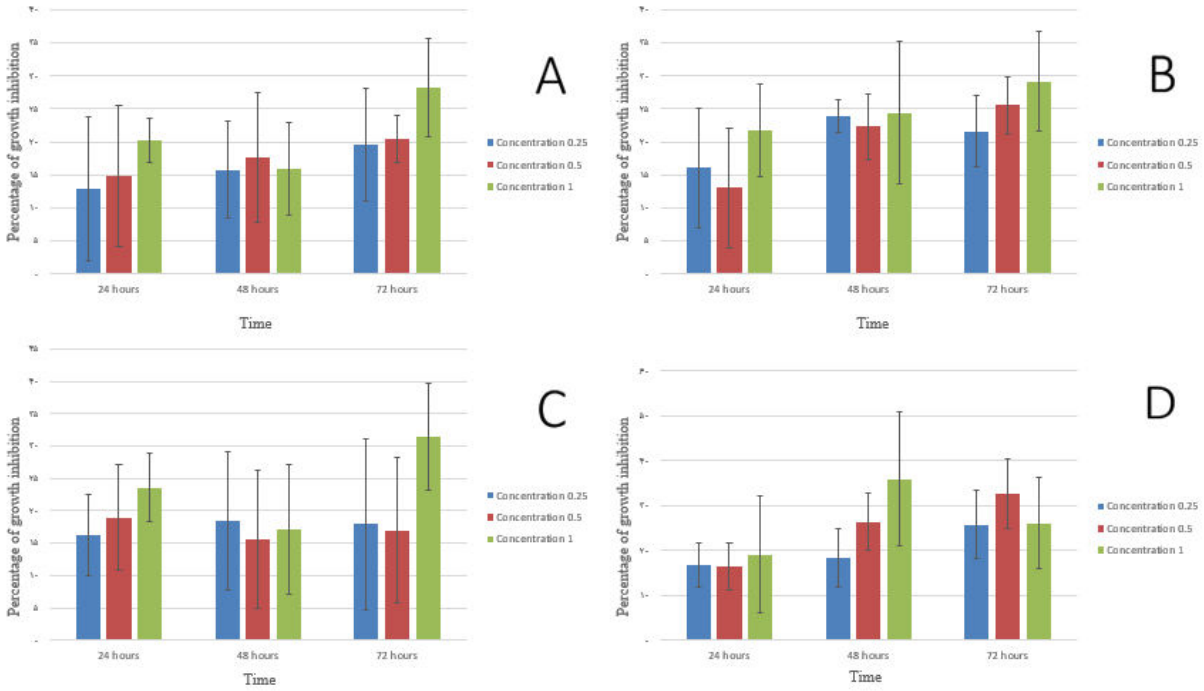


Figure.2 Effect of coelomic fluid concentration on inhibition percentage

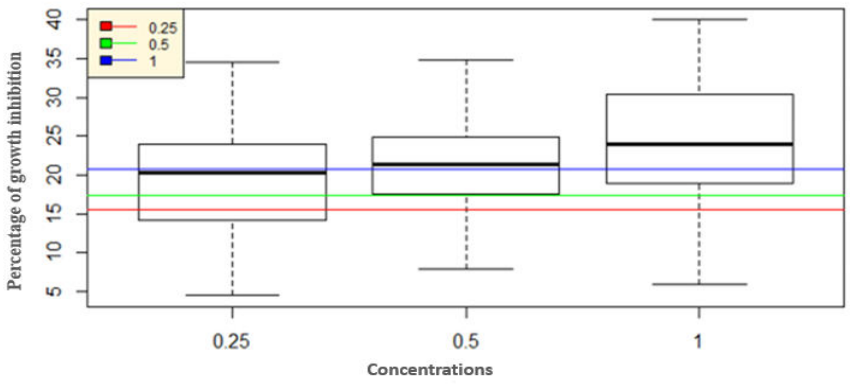


Figure.3 Effect of time on inhibition percentage

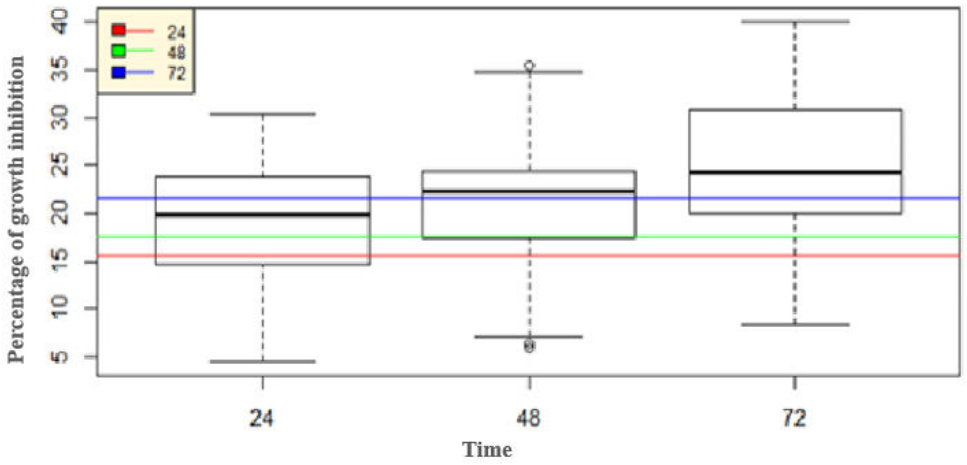


Figure.4 Comparison of inhibition percentage by extraction method

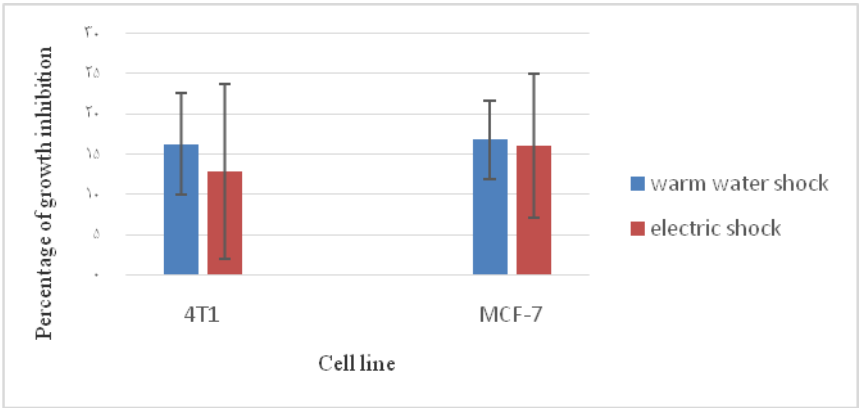


Figure.5 Comparison of inhibition percentage between cell lines

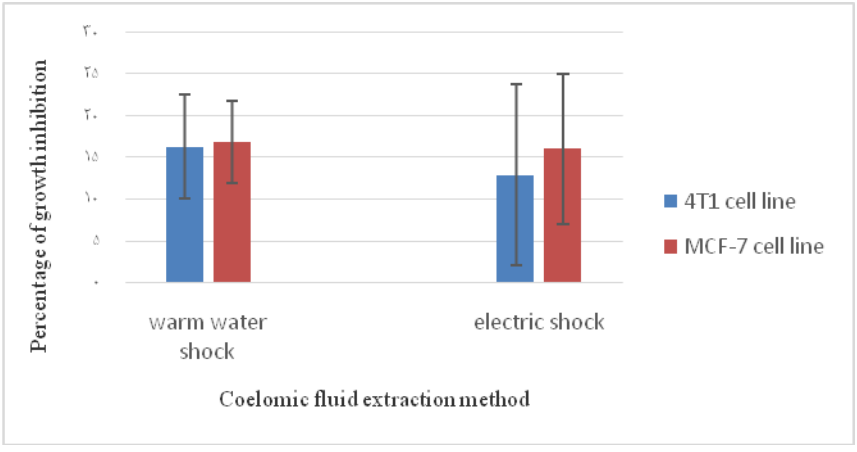
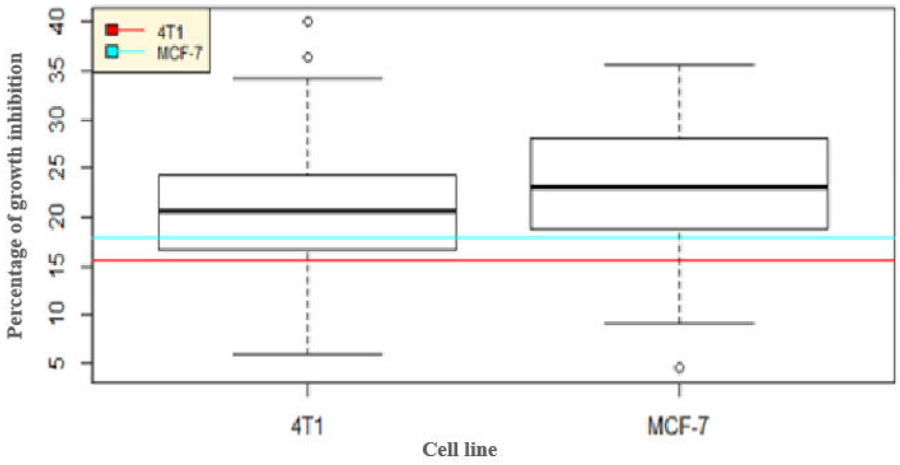


Figure.6 Effect of cell line on inhibition percentage



In a study by Dinesh *et al.,* (2013), peptides extracted from the coelomic fluid of *Eudrilus eugeniae* exhibited cytotoxicity against various cancer cell lines, including HeLa, colon cancer, malignant white blood cell tumors, and brain tumors. Utilizing ice water shock for coelomic fluid collection followed by protein precipitation with different concentrations of ammonium sulfate, the researchers confirmed the anti-tumor efficacy of coelomic fluid proteins (Dinesh *et al.,* 2013).

Augustine *et al.,* (2017) investigated the anti-tumor properties of coelomic fluid extracted via cold shock from *Eisenia fetida*, *Eudrilus eugeniae*, and *Perionyx excavatus* on oral cancer cell lines (SCC-9). Their findings, assessed through MTT assays, underscored the significant antitumor effect of coelomic fluid, with *Eudrilus eugeniae* exhibiting the most potent activity among the tested species (Augustine *et al.,* 2017). Permana *et al.,* (2018) explored the synergistic effect of *Lumbricus rubellus* coelomic fluid in combination with 5-fluorouracil (5-FU) on HT-29 cell lines, revealing inhibition of cell growth and induction of apoptosis.

By combining different concentrations of coelomic fluid with a fixed concentration of 5-FU and evaluating their impact via MTT assays, the researchers highlighted the potential therapeutic synergy of this combination (Permana *et al.,* 2018).

Another study by Augustine *et al.,* (2018) investigated the antitumor effect of coelomic fluid from *Eisenia fetida*, *Eudrilus eugeniae*, and *Perionyx excavatus* on oral cancer cell lines (KB 3-1). Similar to their previous findings, coelomic fluid exhibited notable antitumor activity, with *Eudrilus eugeniae* displaying the most pronounced effect (Augustine *et al.,* 2018).

Fiolka *et al.,* (2019) examined the cytotoxic effects of *Dendrobaena veneta* coelomic fluid on A549 cell lines, observing time- and concentration-dependent reductions in cell viability and altered cell morphology. Additionally, an increase in caspase levels further validated the apoptotic impact of coelomic fluid, as confirmed by flow cytometry analysis (Fiolka *et al.,* 2019).

Permana *et al.,* (2020) explored the anticancer potential of *Eisenia fetida* coelomic fluid in conjunction with cetuximab in colorectal cancer using BALB/c mice models. Their results demonstrated a reduction in K-Ras and vimentin expression, suggesting a promising therapeutic avenue for colorectal cancer treatment (Permana *et al.,* 2020).

Finally, Sadek *et al.,* (2022) conducted a comprehensive investigation into the biological activities of *Allolobophora caliginosa* coelomic fluid (ACCF). Their study revealed the inhibitory effect of coelomic fluid on HepG2 cell proliferation, with an observed IC50 value of 145.99 µg/ml, indicating its potential as a therapeutic agent against hepatocellular carcinoma (Sadek *et al.,* 2022).

In this study, we investigated the extraction methods, protein concentration, and the effect of coelomic fluid from earthworms on cancer cell lines. Our results

demonstrated significant differences between the warm water shock and electric shock methods in terms of coelomic fluid yield and protein concentration. While the electric shock method proved more efficient in fluid extraction, the warm water shock method resulted in higher protein concentration. Both methods exhibited inhibitory effects on MCF-7 and 4T1 cell lines, with the warm water shock method showing slightly superior results in terms of inhibition percentage.

Regression analysis revealed concentration-dependent and time-dependent responses of coelomic fluid on cell line inhibition, with optimal effectiveness observed at lower concentrations and within the initial 24 hours of exposure. Comparative analysis between extraction methods showed comparable inhibitory effects, emphasizing the importance of considering both efficacy and practicality in experimental design.

Our findings contribute to the growing body of research on the anticancer properties of earthworm coelomic fluid. Numerous studies highlighted in the discussion section have explored the cytotoxic effects of coelomic fluid from various earthworm species on different cancer cell lines, further validating its potential as a therapeutic agent against a wide range of cancers.

The apoptotic induction of cancer cells by coelomic fluid, as demonstrated by Yanqin *et al.,* (2007); Dinesh *et al.,* (2013) and Augustine *et al.,* (2017, 2018), underscores its promising anticancer properties.

Moreover, the synergistic effects of coelomic fluid in combination with chemotherapeutic agents, as shown by Permana *et al.,* (2018) and Permana *et al.,* (2020), suggest its potential in enhancing existing cancer treatments.

Additionally, studies such as those by Fiolka *et al.,* (2019) and Sadek *et al.,* (2022) have elucidated the cytotoxic mechanisms of coelomic fluid, providing valuable insights into its mode of action against cancer cells.

Overall, our study contributes to the understanding of earthworm coelomic fluid as a potential therapeutic agent in cancer treatment. Further research is warranted to explore its efficacy in preclinical and clinical settings, as well as its potential in combination therapies and as adjuvants to existing treatments.

Authors' contributions

This article was written entirely by Marzieh Shokoohi, including the design and execution of experiments, data analysis, writing and editing.

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Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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References

"American Cancer Society," *Cancer facts & figures.*, 2008.

"Breast Cancer: What You Need To Know Before Treatment," *NationalCancer Institute.*, pp. 00-1556, 2003.

Allred, D. C. "Ductal carcinoma in situ: terminology, classification, and natural history," *J. Natl. Cancer Inst. Monogr.*, vol. 2010, no. 41, pp. 134–138, 2010.

Ambudkar, S. V., C. Kimchi-Sarfaty, Z. E. Sauna, and M. M. Gottesman, "P-glycoprotein: from genomics to mechanism," *Oncogene*, vol. 22, no. 47, pp. 7468–7485, 2003. https://doi.org/10.1038/sj.onc.1206948

Augustine, D., R. Rao, A. Jayaraman, and K. N. Chidambara Murthy, "Anti-proliferative activity of earthworm coelomic fluid using oral squamous carcinoma KB 3-1 cells: An *In vitro* study with serine protease analysis," *Pharmacogn. Mag.*, vol. 14, no. 59, p. 528, 2018. https://doi.org/10.4103/pm.pm_412_18

Augustine, D., R. Rao, J. Anbu, and K. N. Chidambara Murthy, "Anticancer prospects of earthworm extracts: A systematic review of *In vitro* and In vivo studies," *Pharmacogn. Rev.*, vol. 12, no. 23, p. 46, 2018.

Augustine, D., R. Rao, J. Anbu, and K. N. Chidambara Murthy, "*In vitro* antiproliferative effect of earthworm coelomic fluid of *Eudrilus eugeniae*, *Eisenia foetida*, and *Perionyx excavatus* on squamous cell carcinoma-9 cell line: A pilot study," *Pharmacognosy Res.*, vol. 9, no. 5, p. 61, 2017. https://doi.org/10.4103/pr.pr_52_17

Augustine, D., R. S. Rao, J. Anbu, and K. N. Chidambara Murthy, "*In vitro* cytotoxic and apoptotic induction effect of earthworm coelomic fluid of *Eudrilus eugeniae*, *Eisenia foetida*, and *Perionyx excavatus* on human oral squamous cell carcinoma-9 cell line," *Toxicol. Rep.*, vol. 6, pp. 347–357, 2019. https://doi.org/10.1016/j.toxrep.2019.04.005

Bernardini, S., A. Tiezzi, V. LaghezzaMasci, and E. Ovidi, "Natural products for human health: an historical overview of the drug discovery approaches," *Nat. Prod. Res.*, vol. 32, no. 16, pp. 1926–1950, 2018.

Bradford, M. M, "A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding," *Analytical biochemistry.*, vol. *72*, no. (1-2), pp. 248-254, 1976. https://doi.org/10.1006/abio.1976.9999

Danaei, G., S. Vander Hoorn, A. D. Lopez, C. J. L. Murray, M. Ezzati, and Comparative Risk Assessment collaborating group (Cancers), "Causes of cancer in the world: comparative risk assessment of nine behavioural and environmental risk factors," *Lancet*, vol. 366, no. 9499, pp. 1784–1793, 2005. https://doi.org/10.1016/S0140-6736(05)67725-2

de Martel C. *et al.,* "Global burden of cancers attributable to infections in 2008: a review and synthetic analysis," *Lancet Oncol.*, vol. 13, no. 6, pp. 607–615, 2012. https://doi.org/10.1016/S1470-2045(12)70137-7

Dinesh, M. S., S. Sridhar, P. G. Chandana, V. Pai, K. S. Geetha, and R. N. Hegdge, "Anticancer Potentials of Peptides of Coelomic Fluid of Earthworm *Eudrilus eugeniae*," *Biosci. Biotechnol. Res. Asia*, vol. 10, no. 2, pp. 601–606, 2013.

Dittmer, J., I. Oerlecke, and B. Leyh, "Involvement of mesenchymal stem cells in breast cancer progression," in *Breast Cancer - Focusing Tumor Microenvironment, Stem cells and Metastasis*, InTech, 2011. https://doi.org/10.5772/21325

DuPré, S. A., D. Redelman, and K. W. Hunter Jr, "The mouse mammary carcinoma 4T1: characterization of the cellular landscape of primary tumours and metastatic tumour foci: Cellular landscape of mammary carcinoma 4T1," *Int. J. Exp. Pathol.*, vol. 88, no. 5, pp. 351–360, 2007. https://doi.org/10.1111/j.1365-2613.2007.00539.x

Edwards, C. A. and P. J. Bohlen, "Biology and ecology of earthworms," *Biology and ecology of earthworms*, vol. 3, 1996.

Fiaschi, T. and P. Chiarugi, "Oxidative stress, tumor microenvironment, and metabolic reprogramming: a diabolic liaison," *Int. J. Cell Biol.*, vol. 2012, p. 762825, 2012. https://doi.org/10.1155/2012/762825

Fiołka, Marta J., Jolanta Rzymowska, SylwiaBilska, KingaLewtak, Magdalena Dmoszyńska-Graniczka, Krzysztof Grzywnowicz, Wojciech Kaźmierski, and Teresa Urbanik-Sypniewska. "Antitumor activity and apoptotic action of coelomic fluid from the earthworm *Dendrobaena veneta* against A549 human lung cancer cells," *Apmis* 127.,vol. 127, no. 9, pp. 435-448, 2019. https://doi.org/10.1111/apm.12941

Gottesman, M. M. "How cancer cells evade chemotherapy: sixteenth Richard and Hinda Rosenthal Foundation Award Lecture," *Cancer Res.*, vol. 53, no. 4, pp. 747–754, 1993.

Horwitz, K. B., M. E. Costlow, and W. L. McGuire, "MCF-7: A human breast cancer cell line with estrogen, androgen, progesterone, and glucocorticoid receptors," *Steroids*, vol. 26, no. 6, pp. 785–795, 1975. https://doi.org/10.1016/0039-128x(75)90110-5

Irma, J. H. and J. H. Beijnen, "Combined action and regulation of phase II enzymes and multidrug resistance proteins in multidrug resistance in cancer," *Cancer treatment reviews*, vol. 34, pp. 505–520, 2008.

Macdonald, F., C. Ford, and A. Casson, *Molecular biology of cancer*, 2nd ed. London, England:

Taylor & Francis, 2004. https://doi.org/10.4324/9780203503447

McGuire, S., "World cancer report 2014. Geneva, Switzerland: World health organization, international agency for research on cancer, WHO press, 2015," *Adv. Nutr.*, vol. 7, no. 2, pp. 418–419, 2016. https://doi.org/10.3945/an.116.012211

Nelson, N. J. "Migrant studies aid the search for factors linked to breast cancer risk," *J. Natl. Cancer Inst.*, vol. 98, no. 7, pp. 436–438, 2006.

Nicholas Zdenkowski, P. Butow, S. Tesson, and F. Boyle, "A systematic review of decision aids for patients making a decision about treatment for early breast cancer," *Breast*, vol. 26, pp. 31–45, 2016. https://doi.org/10.1016/j.breast.2015.12.007

Patil, S. R. and P. M. Biradar, "Earthworm's coelomic fluid: extraction and importance," *International Journal of Advanced Scientific Research*, vol. 2, no. 2, pp. 1–04, 2017.

Permana *et al.,* (2018) "Cytotoxic effects and anti-proliferative cancer activity of coelomic fluid from *Lumbricus rubellus* promotes apoptosis and reduces G2/M phase progression in HT-29 cells," *J. Appl. Pharm. Sci.*, vol. 8, no. 11, pp. 28–34, 2018. https://doi.org/10.7324/JAPS.2018.81104

Permana, Sofy, Reyudzky Putri Fityanti, Eviana Norahmawati, Agustin Iskandar, Erika Desy Anggraini Mulyadi, and Agustina Tri Endharti. "Coelomic Fluid of *Eisenia fetida* Ameliorates Cetuximab to Reduce K-Ras and Vimentin Expression through Promoting RUNX3 in an AOM/DSS-Induced Colitis Associated Colon Cancer." *Evidence-Based Complementary and Alternative Medicine* 2020.,2020. https://doi.org/10.1155/2020/9418520

Porter, P. L. "Global trends in breast cancer incidence and mortality," *SaludPublica Mex.*, vol. 51, pp. s141–s146, 2009.

Rathi, S. G. "In-vitro cytotoxic screening of *Glycyrrhiza glabra* L. (Fabaceae): A natural anticancer drug," *Journal of Young Pharmacists*, vol. 1, no. 3, 2009. https://doi.org/10.4103/0975-1483.57071

Sadek, Shimaa A., Moshera G. Sayed, Sohair R. Fahmy, and Amel M. Soliman. "A Coelomic Fluid of *Allolobophora caliginosa* as Novel Prospects for Medicinal Antioxidants, Anti-inflammatory, Antiproliferative, Analgesics, and Antipyretics." 2022.

Schweizer, R., W. Tsuji, V. S. Gorantla, K. G. Marra, J. P. Rubin, and J. A. Plock, "The role of adipose-derived stem cells in breast cancer progression and metastasis," *Stem Cells Int.*, vol. 2015, p. 120949, 2015. https://doi.org/10.1155/2015/120949

Sekhar, K. C., A. Rajanikanth, M. N. Bobby, and J. R. Kanala, "A review on anticancer potential of natural drugs: Hispolon and limonene," *Int. J. Curr. Microbiol. Appl. Sci.*, vol. 7, no. 11, pp. 3253–3263, 2018.

Sethulakshmi, K. and R. Lakshmi, "Antibacterial activities of coelomic fluid of local earthworms against disease causing microorganisms," *Asian J. Biol.*, vol. 5, no. 3, pp. 1–7, 2018.

Sudhakar, A. "History of cancer, ancient and modern treatment methods," *J. Cancer Sci. Ther.*, vol. 1, no. 2, pp. 1–4, 2009. https://doi.org/10.4172/1948-5956.100000e2

Sun, Z., "Earthworm as a biopharmaceutical: from traditional to precise," *Eur. j. biomed. res.*, vol. 1, no. 2, pp. 28-35, 2015. https://doi.org/10.18088/ejbmr.1.2.2015.pp28-35

Urry, L. A., M. L. Cain, S. A. Wasserman, P. V. Minorsky, and J. B. Reece, *Campbell biology in focus*, 2nd ed. Upper Saddle River, NJ: Pearson, 2015.

Wu, S., S. Powers, W. Zhu, and Y. A. Hannun, "Substantial contribution of extrinsic risk factors to cancer development," *Nature*, vol. 529, no. 7584, pp. 43–47, 2016. https://doi.org/10.1038/nature16166

Yanqin L. *et al.,* "Coelomic fluid of the earthworm *Eisenia fetida* induces apoptosis of HeLa cells *In vitro*," *Eur. J. Soil Biol.*, vol. 43, pp. S143–S148, 2007. https://doi.org/10.1016/j.ejsobi.2007.08.049

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